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(FILE 'HOME' ENTERED AT 14:23:28 ON 15 SEP 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 14:23:44 ON 15 SEP 2003

L1 2838 S SKIN(W)SUBSTITUTE OR SKIN(3A)EQUIVALEN?
L2 28417 S CERAMIDE
L3 42 S L1 AND L2
L4 14014 DUP REM L2 (14403 DUPLICATES REMOVED)
L5 20 DUP REM L3 (22 DUPLICATES REMOVED)
L6 1838 S CERAMIDE(5A) (5 OR 6 OR 7)
L7 3 S L5 AND L6
L8 3 DUP REM L7 (0 DUPLICATES REMOVED)

=> d au ti so ab 1-3 l8

L8 ANSWER 1 OF 3 MEDLINE on STN
AU Vicanova J; Boyce S T; Harriger M D; Weerheim A M; Bouwstra J A; Ponec M
TI Stratum corneum lipid composition and structure in cultured **skin substitutes** is restored to normal after grafting onto athymic mice.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY. SYMPOSIUM PROCEEDINGS, (1998 Aug) 3 (2) 114-20.
Journal code: 9609059. ISSN: 1087-0024.
AB Restoration of an epidermal barrier is a definitive requirement for wound closure. Cultured **skin substitutes** grafted onto athymic nude mice were used as a model for a long-term study of stratum corneum barrier lipid metabolism and organization. Samples of stratum corneum collected after 12 and 21 d in vitro and 6, 11, and 24 mo postgrafting were examined for their lipid and fatty acid composition, and their lipid organization and structure using electron microscopy and small angle X-ray diffraction, respectively. All of these methods confirm the impaired barrier function of cultured **skin substitutes** in vitro, as judged from the deviations in lipid composition and from poor organization of the stratum corneum lipids that show no lamellar structure. At 6 mo postgrafting, the total stratum corneum lipid profiles of the epidermal grafts is close to that of the human stratum corneum with the exception of the presence of mouse specific lipids. The increase of **ceramides** 4-7 in cultured **skin substitutes** after grafting indicates restored activity of processes involved in the hydroxylation of fatty acids and sphingoid bases. Conversely, the **ceramide** profile still reveals some abnormalities (elevated content of **ceramide** 2 and slightly lower content of **ceramide** 3) and the content of long-chain fatty acids remains below its physiologic level at 6 mo postgrafting, but normalizes by 2 y postgrafting. The ultramicroscopic observations revealed the formation of lamellar extracellular lipid domains by 4 mo postgrafting. Despite these findings, the X-ray diffraction showed differences in the diffraction pattern at 2 y after grafting, suggesting that the organization of stratum corneum lipids in all epidermal grafts differs from that of the native skin.

L8 ANSWER 2 OF 3 MEDLINE on STN
AU Ponec M; Weerheim A; Kempenaar J; Mulder A; Gooris G S; Bouwstra J; Mommaas A M
TI The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1997 Sep) 109 (3) 348-55.
Journal code: 0426720. ISSN: 0022-202X.
AB Our analysis of epidermal lipids revealed that (glucosyl)**ceramide** profiles in various human **skin equivalents** are different from those of native tissue. The main difference is the reduced

content in **skin equivalents** of **ceramides** 4-7 and especially the very low content of the most polar **ceramides** 6 and 7, which contain hydroxylated sphingoid base and/or fatty acid. To facilitate hydroxylation, the culture medium was supplemented with vitamins C and E. Although in vitamin E-supplemented medium lipogenesis was not affected, in vitamin C-supplemented medium the content of glucosylceramides and of **ceramides** 6 and 7 was markedly increased, both in the presence and absence of serum and irrespective the substrate used (inert or natural, populated or not with fibroblasts). The improvement of the lipid profile was accompanied by a marked improvement of the barrier formation as judged from extensive production of lamellar bodies, their complete extrusion at the stratum granulosum/stratum corneum interface, and the formation of multiple broad lipid lamellar structures in the intercorneocyte space. The presence of well-ordered lipid lamellar phases was confirmed by small-angle x-ray diffraction. Some differences between native and reconstructed epidermis, however, were noticed. Although the long-range lipid lamellar phase was present in both the native and the reconstructed epidermis, the short lamellar phase was present only in native tissue. It remains to be established whether these differences can be ascribed to small differences in relative amounts of individual **ceramides**, to differences in fatty acid profiles, or to differences in cholesterol sulfate, pH, or calcium gradients. The results indicate the key role vitamin C plays in the formation of stratum corneum barrier lipids.

- L8 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AU Nolte, C. J. M. (1); Oleson, M. A.; Bilbo, P. R.; Parenteau, N. L.
 TI Development of a stratum corneum and barrier function in an organotypic skin culture.
 SO Archives of Dermatological Research, (1993) Vol. 285, No. 8, pp. 466-474. ISSN: 0340-3696.
 AB The stratum corneum of human skin is responsible for maintaining the epidermal permeability barrier. We have developed a bilayered skin culture (SC) which forms a corneum 35 +/- 1 cell layers thick 21 days after being raised to the air-liquid (A/L) interface. By the 7th day after raising to the A/L interface the corneocytes were irregularly shaped and had cross-sectional areas (CSA) of $300 \mu\text{m}^2$. By the 21st day the corneocytes had assumed polygonal shapes and had a CSA (100-250 μm^2) similar to that of human foreskin. The total lipid (TL) content of the corneum averaged 5-7% of the lyophilized weight. **Ceramide** content increased from 20% of TL at day 7 of A/L interface culture to 30% at day 21. Triglycerides decreased from 43% to 17% of TL during the same period. Free fatty acids comprised 5.5% of TL at day 21 of A/L interface culture. The intercorneocyte spaces contained stacks of lipid lamellae. However, the stacks lacked the Landmann unit repeat. Abnormal lamellar structures were observed in both the intra- and extracorneocyte spaces. Transepidermal water loss (TEWL) was $> 4 \text{ mg/cm}^2$ per h throughout the culture period. Lipid supplementation of the culture medium and culturing in a low humidity environment improved barrier function by 50%. However, the effects were not additive. The SC developed a near-normal corneum, but did not achieve barrier competence, due at least partially to abnormalities in lipid composition and organization. Improvement of barrier function with lipid supplementation or low humidity indicates that modifications of the culture environment may facilitate the SC in assembling a permeability barrier **equivalent** to human skin.

=> d au ti so ab 1-20 15

- L5 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
 AU Martini, M. C.
 TI Biochemical analysis of epidermal lipids

SO Pathologie Biologie (2003), 51(5), 267-270

CODEN: PTBIAN; ISSN: 0369-8114

AB La compn. des lipides cutane.acts e.acte.tant de.acte.ja` connue, les me.acte.thodes analytiques de.acte.veloppe.acte.es re.acte.cemment tendent pluto.cxa.t a` pre.acte.ciser l'organization structurale de ces diffe.acte.rents e.acte.le.acte.ments de fac.cdll.on a` pouvoir e.acte.lucider les me.acte.canismes contro.cxa.lant la fonction barriere et la desquamation et en conse.acte.quence intervenant dans l'hydratation et la fixation ou l'absorption percutane.acte.e des substances exoge`nes. Plusieurs auteurs utilisent la microspectroscopie Raman ou la diffraction des rayons X, la calorime.acte.trie diffe.acte.rentielle et la spectroscopie infrarouge a` transforme.acte.e de Fourier en plus des me.acte.thodes classiques comme l'HPTLC, la chromatographie sur couche mince ou sur baguettes de silice. A` la lumie`re des re.acte.sultats obtenus, il s'ave`re que la connaissance de l'organization structurale est primordiale pour expliquer en particulier les phe.acte.nome`nes lie.acte.s a` la perme.acte.abilite.acte. cutane.acte.e qu'ils soient physiologiques ou pathologiques. Les implications pratiques concernent aussi bien la the.acte.rapie topique que la cosme.acte.tologie. According to the knowledge acquired some 15 yr ago, the cutaneous lipids may be classified into 2 families: the "neutral" lipids, represented by cholesterol, cholesterol esters, cholesterol sulfate, triglycerides, free fatty acids, squalen and alkanes, and the "polar" lipids including phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingomyeline) and sphingolipids (**ceramides** I-VII, monohexosylceramides). From the functional point of view, free fatty acids, cholesterol, and **ceramides** organised in layers are the most important components of intercellular spaces of the stratum corneum. Analytic methods have been recently developed to help understand the structural organization of these various mols. within the horny layer and their influence on the epidermal barrier function. Raman microspectroscopy or X-ray diffraction are most frequently used. Differential calorimetry and fluorescence or IR spectroscopy provide complementary information. The principal findings are: lamellar structure depends on the presence of **ceramides** supplemented by adequate quantities of free fatty acids and cholesterol;**ceramide** chains interact to provide the ordered structure and **ceramide-1** is necessary for stabilization of lipid layers;cholesterol may regulate the mol. mobility of hydrocarbon chains within the bi-layers.Knowledge of the mol. structure of the barrier lipids finds several applications, e.g.: in pharmacol.-conception of new formulations adapted for percutaneous and topical application of drugs; in dermatol.-comprehension of physiopathol. mechanisms of various dermatoses; in biotechnol.-development of **skin substitutes** with valid stratum corneum barrier; in cosmetics-choice of best formulations suited for reconstruction of the intercellular lipid substance.

L5 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN

IN Comer, Allen; Allen-Hoffmann, Lynn; Hoffmann, Michael; Ivarie, Cathy Ann-Rasmussen; Conrad, Paul Barth

TI Improved **skin substitutes** and uses thereof

SO PCT Int. Appl., 104 pp.

CODEN: PIXXD2

AB The present invention relates to in vitro cultured **skin substitutes**, and in particular to in vitro cultured **skin substitutes** that have improved barrier function. In some embodiments, improved barrier function is a result of improved culture conditions, while in other embodiments, improved barrier function results from genetic modification of keratinocyte. Improved culture conditions to improve barrier function include organotypic culture in the presence of linoleic acid and/or linoleic acid at about 75% humidity. Suitable genetic modifications for improving barrier function includes transfection with a DNA construct capable of expressing GKLF. The present invention further provides improved methods of using such **skin**

substitutes in the screening of compds. for irritancy activity, as well for identifying novel irritant responsive genes.

- L5 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
IN Philippe, Michel; Castiel, Isabelle; Ferraris, Corinne; Arbey, Eric
TI Cosmetic composition comprising a **ceramide** precursor for
improving natural or reconstructed epidermis, resulting **skin**
equivalent
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
AB The invention concerns a compn. comprising, in a physiol. acceptable medium, at least a **ceramide** precursor, the use of said compd. in particular for reinforcing the lipid barrier of the epidermis and/or for improving the quality and properties of reconstructed epidermis, and a **skin equiv.** supplemented with said compd. A moisturizer oil-in-water emulsion contained maize germ oil 2, glycerol monostearate 3, polyethylene glycol 3, gelling agents 0.2, iso-Pr myristate 3.0, 6-OH-sphingenine 0.1, cetyl alc. 3, stearic alc. 3, sodium hydroxide 0.008, propylene glycol 5.0, preservatives q.s., and water q.s. 100 g.
- L5 ANSWER 4 OF 20 MEDLINE on STN DUPLICATE 1
AU Boxman Ingeborg L A; Kempenaar Johanna; de Haas Ellis; Ponc Maria
TI Induction of HSP27 nuclear immunoreactivity during stress is modulated by vitamin C.
SO EXPERIMENTAL DERMATOLOGY, (2002 Dec) 11 (6) 509-17.
Journal code: 9301549. ISSN: 0906-6705.
AB For the investigation of the skin irritancy potential of chemicals in an in vitro model it is necessary to have sensitive endpoints that predict the effects of those compounds on native human skin. Recently, we have identified that 27-kDa heat shock protein (HSP27) can serve as a sensitive marker of skin irritation, as exposure of human skin to sodium lauryl sulfate (SLS) both in vitro and in vivo induced relocalization of HSP27 from the cytoplasm to the cell nucleus. The aim of the present study was to determine whether nuclear localization of HSP27 could be used as a parameter for evaluation of potential skin irritants in screening assays in vitro. For this purpose, human **skin equivalent** consisting of epidermis reconstructed on de-epidermized dermis was exposed to SLS or UV light. Stress-induced nuclear relocalization of HSP27 was observed in excised skin exposed to SLS or UV light and in reconstructed epidermis only when the latter was generated in the absence of vitamin C. The omission of vitamin C results in an impaired barrier function. In the presence of vitamin C, however, the barrier function was comparable with excised skin, suggesting that vitamin C may control the response to stress in the reconstructed epidermis. Besides the presence of vitamin C, the response of **skin equivalents** may strongly depend on other conditions under which they are generated, because the stress-induced HSP27 relocalization was not detected in the commercially available epidermal kit EpiDerm. The results of the present study show that HSP27 nuclear staining can serve as a sensitive marker for skin irritation or cellular stress in excised skin as well as in certain well-characterized human **skin equivalents** in vitro.
- L5 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
IN Castiel, Isabelle; Leclerc, Christelle; Ferraris, Corinne
TI Method for evaluating the effect of a product on epidermal lipogenesis
SO Fr. Demande, 14 pp.
CODEN: FRXXBL
AB A method is provided for evaluation of the effect of a product on epidermal lipogenesis, the method using an **equiv.** of **skin** reconstituted in vitro. the effect of propylene glycol and of N-octylaminocarbonyl-N-methyl-D-glucamine on epidermal lipogenesis was detd.
- L5 ANSWER 6 OF 20 MEDLINE on STN DUPLICATE 2

AU Rivier M; Castiel I; Safonova I; Ailhaud G; Michel S
 TI Peroxisome proliferator-activated receptor-alpha enhances lipid metabolism in a **skin equivalent** model.
 SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (2000 Apr) 114 (4) 681-7.
 Journal code: 0426720. ISSN: 0022-202X.

AB Peroxisome proliferator-activated receptors are involved in certain cell types such as adipocytes and hepatocytes, in the control of several pathways of lipid synthesis or catabolism by regulating the gene expression level of key lipid metabolizing enzymes. As the epidermis exhibits an extensive lipid metabolism necessary for the establishment of the barrier function, we have examined the role of peroxisome proliferator-activated receptor-alpha activation in this process. Living **skin equivalents** were treated with Wy 14,643, a selective peroxisome proliferator-activated receptor-alpha ligand, which enhanced greatly the synthesis of membrane coating granules, the organelles specialized in the processing of stratum corneum lipids. Also, the overall stratum corneum neutral lipid content assessed by Oil red O staining was increased. A detailed analysis of the lipid species present in the reconstructed epidermis showed that peroxisome proliferator-activated receptor-alpha activation increased the synthesis of **ceramides** and cholesterol derivatives, thought to be essential structural components of the permeability barrier. A synergistic effect was observed on lipid synthesis when peroxisome proliferator-activated receptor-alpha and retinoid X receptor were simultaneously activated by selective ligands. Furthermore, activation of peroxisome proliferator-activated receptor-alpha led to increased mRNA expression of several key enzymes of **ceramide** and cholesterol metabolism. An increase of serine-palmitoyl transferase and of beta-glucocerebrosidase enzymatic activity was also demonstrated. Altogether, these results show that peroxisome proliferator-activated receptor-alpha is a key transcription factor involved in the control of the epidermal lipid barrier.

L5 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
 AU Asbill, C.; Kim, N.; El-Kattan, A.; Wertz, P.; Michniak, B.
 TI Lipid composition of a bioengineered human **skin equivalent**
 SO Proceedings of the International Symposium on Controlled Release of Bioactive Materials (2000), 27th, 253-254
 CODEN: PCRMEY; ISSN: 1022-0178

AB The lipid compn. of a novel bioengineered human skin (BHS) was examd. in order to access its utility as an in vitro model for transdermal/topical formulation testing. The lipid compn. of the BHS contained all of the lipids present in human skin. However, the compn. was different with triglycerides making up the majority of lipids found in the BHS. The polar **ceramides** were underrepresented in the BHS while the nonpolar **ceramides** were overrepresented.

L5 ANSWER 8 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AU Rivier, M. (1); Castiel, I.; Safonova, I. (1); Ailhaud, G.; Michel, S. (1)
 TI Peroxisome proliferator-activated receptor-01alpha (PPAR-01alpha) enhances lipid metabolism in a **skin equivalent** model.
 SO Journal of Investigative Dermatology, (April, 1999) Vol. 112, No. 4, pp. 605.
 Meeting Info.: 60th Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 5-9, 1999
 ISSN: 0022-202X.

L5 ANSWER 9 OF 20 MEDLINE on STN DUPLICATE 3
 AU Boelsma E; Verhoeven M C; Ponc M
 TI Reconstruction of a human **skin equivalent** using a spontaneously transformed keratinocyte cell line (HaCaT).
 SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1999 Apr) 112 (4) 489-98.
 Journal code: 0426720. ISSN: 0022-202X.

AB Reconstruction of a **skin equivalent** using an immortalized human keratinocyte line, HaCaT, was investigated in an attempt to generate an in vitro system representative for human skin. Three different substrates were used to establish air-exposed cultures of HaCaT cells: de-epidermized dermis, collagen gels, and filter inserts. Effects of variations in culture conditions on tissue morphology, on the expression of proliferation-specific and differentiation-specific protein markers, and on lipid profiles were investigated. When grown at the air-liquid interface HaCaT cells initially developed a multilayered epithelium, but during the course of culture marked alterations in tissue architecture were observed. Ultrastructurally, a disordered tissue organization was evident as judged from the presence of rounded cells with abnormally shaped nuclei. Keratins K1 and K10 were irregularly expressed in all cell layers, including stratum basale. Staining of K6/K16 was evident in all cell layers. Locally, basal and suprabasal cells were positive for K4 and additionally expressed K13 and K19. The cornified envelope precursors were expressed only in older cultures (>2 wk after air exposure), except for transglutaminase and small proline rich protein 1, which were irregularly expressed in both early and older cultures. In addition, HaCaT cells showed an impaired capacity to synthesize lipids that are necessary for a proper barrier formation as indicated by the absence of free fatty acids and a very low content and incomplete profile of **ceramides**. Our data demonstrate that the ultimate steps of terminal differentiation in HaCaT cells do not occur irrespective of the type of substrate or the culture conditions.

L5 ANSWER 10 OF 20 MEDLINE on STN DUPLICATE 4
AU Robert M; Bissonauth V; Ross G; Rouabhia M
TI Harmful effects of UVA on the structure and barrier function of engineered human cutaneous tissues.
SO INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, (1999 Mar) 75 (3) 317-26. Journal code: 8809243. ISSN: 0955-3002.
AB PURPOSE: To investigate the effects of a single UVA exposure on engineered human cutaneous tissues. MATERIALS AND METHODS: **Skin equivalents** (SE) were obtained by culturing keratinocytes on fibroblast-populated collagen gels; epidermal equivalents (EE) were obtained by seeding keratinocytes on non-populated collagen gels. After maturation and differentiation of the epidermis, SE and EE were exposed to 50 or 100 J/cm² UVA. Structural damage and total epidermal lipids were analysed and diffusion of radioactive oestradiol was monitored 24 and 72h post-irradiation. RESULTS: Twenty-four hours after UVA irradiation, a disorganization of the living epidermis is observed. UVA also significantly reduced the skin barrier function and led to an increase in phospholipid and in a decrease of **ceramide** levels. However, both the structure and the barrier function of SE were recovered 72 h post-irradiation, thereby suggesting that an intrinsic repair process might exist within the irradiated SE. CONCLUSION: This study provides a strong evidence that UVA radiation alters both the epidermal and dermal structures, the synthesis of epidermal lipids, and the permeability of the human skin.

L5 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
AU Chouinard, Nadine; Rouabhia, Mahmoud
TI Effects of all-trans retinoic acid on UVB-irradiated human **skin substitute**
SO Journal of Cellular Physiology (1999), 181(1), 14-23 CODEN: JCLLAX; ISSN: 0021-9541
AB Retinoids are frequently used for treatment of photodamaged skin. We wished to find out whether photodamage could be attenuated by applying all-trans retinoic acid (RA) during repetitive irradiation. For this purpose, we used human cutaneous cells and tissue: pure monolayer cultures containing either keratinocytes or fibroblasts, and human **skin substitute** (SS) containing both cell types. All cultures were exposed to 8 mJ/cm² of UVB and were immediately treated with RA (0, 1.5, or 3

.mu.M). The irradiation and RA treatment protocol was repeated until the cells of the nonirradiated culture had reached confluence. In the irradiated SS, RA preserved the structure (epidermal stratification and differentiation) and ultra-structure (well-organized intermediate filaments and desmosomes) in a state comparable to that observed in nonirradiated SS. As well, RA maintained secretion of basement membrane components (laminin and type-IV collagen). Following irradiation, cutaneous cells also displayed more proliferative capacity when SS was treated. In the irradiated monolayer cultures, RA maintained the proliferative capacity of fibroblasts and decreased their differentiation whereas the opposite effect was seen on keratinocytes. In conclusion, RA clearly helps protect human skin against photodamage induced by repeated exposure to UVB.

- L5 ANSWER 12 OF 20 MEDLINE on STN DUPLICATE 5
 AU Vicanova J; Boyce S T; Harriger M D; Weerheim A M; Bouwstra J A; Ponc M
 TI Stratum corneum lipid composition and structure in cultured **skin substitutes** is restored to normal after grafting onto athymic mice.
 SO JOURNAL OF INVESTIGATIVE DERMATOLOGY. SYMPOSIUM PROCEEDINGS, (1998 Aug) 3 (2) 114-20.
 Journal code: 9609059. ISSN: 1087-0024.
 AB Restoration of an epidermal barrier is a definitive requirement for wound closure. Cultured **skin substitutes** grafted onto athymic nude mice were used as a model for a long-term study of stratum corneum barrier lipid metabolism and organization. Samples of stratum corneum collected after 12 and 21 d in vitro and 6, 11, and 24 mo postgrafting were examined for their lipid and fatty acid composition, and their lipid organization and structure using electron microscopy and small angle X-ray diffraction, respectively. All of these methods confirm the impaired barrier function of cultured **skin substitutes** in vitro, as judged from the deviations in lipid composition and from poor organization of the stratum corneum lipids that show no lamellar structure. At 6 mo postgrafting, the total stratum corneum lipid profiles of the epidermal grafts is close to that of the human stratum corneum with the exception of the presence of mouse specific lipids. The increase of **ceramides** 4-7 in cultured **skin substitutes** after grafting indicates restored activity of processes involved in the hydroxylation of fatty acids and sphingoid bases. Conversely, the **ceramide** profile still reveals some abnormalities (elevated content of **ceramide** 2 and slightly lower content of **ceramide** 3) and the content of long-chain fatty acids remains below its physiologic level at 6 mo postgrafting, but normalizes by 2 y postgrafting. The ultramicroscopic observations revealed the formation of lamellar extracellular lipid domains by 4 mo postgrafting. Despite these findings, the X-ray diffraction showed differences in the diffraction pattern at 2 y after grafting, suggesting that the organization of stratum corneum lipids in all epidermal grafts differs from that of the native skin.
- L5 ANSWER 13 OF 20 MEDLINE on STN DUPLICATE 6
 AU Ponc M; Weerheim A; Kempenaar J; Mulder A; Gooris G S; Bouwstra J; Mommaas A M
 TI The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C.
 SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1997 Sep) 109 (3) 348-55.
 Journal code: 0426720. ISSN: 0022-202X.
 AB Our analysis of epidermal lipids revealed that (glucosyl)**ceramide** profiles in various human **skin equivalents** are different from those of native tissue. The main difference is the reduced content in **skin equivalents** of **ceramides** 4-7 and especially the very low content of the most polar **ceramides** 6 and 7, which contain hydroxylated sphingoid base and/or fatty acid. To facilitate hydroxylation, the culture medium was supplemented with

vitamins C and E. Although in vitamin E-supplemented medium lipogenesis was not affected, in vitamin C-supplemented medium the content of glucosylceramides and of **ceramides** 6 and 7 was markedly increased, both in the presence and absence of serum and irrespective the substrate used (inert or natural, populated or not with fibroblasts). The improvement of the lipid profile was accompanied by a marked improvement of the barrier formation as judged from extensive production of lamellar bodies, their complete extrusion at the stratum granulosum/stratum corneum interface, and the formation of multiple broad lipid lamellar structures in the intercorneocyte space. The presence of well-ordered lipid lamellar phases was confirmed by small-angle x-ray diffraction. Some differences between native and reconstructed epidermis, however, were noticed. Although the long-range lipid lamellar phase was present in both the native and the reconstructed epidermis, the short lamellar phase was present only in native tissue. It remains to be established whether these differences can be ascribed to small differences in relative amounts of individual **ceramides**, to differences in fatty acid profiles, or to differences in cholesterol sulfate, pH, or calcium gradients. The results indicate the key role vitamin C plays in the formation of stratum corneum barrier lipids.

- L5 ANSWER 14 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AU Vicanova, Jana; Ponec, Maria (1); Weerheim, Arij; Swope, Viki; Westbrook, Melissa; Harriger, Dana; Boyce, Steven
 TI Epidermal lipid metabolism of cultured **skin substitutes** during healing of full-thickness wounds in athymic mice.
 SO Wound Repair and Regeneration, (Oct.-Dec., 1997) Vol. 5, No. 4, pp. 329-338.
 ISSN: 1067-1927.
- AB Cultured epidermal keratinocytes provide an abundant supply of biologic material for wound treatment. Because restoration of barrier function is a definitive criterion for efficacy of wound closure and depends on the lipids present in the epidermis, we analyzed lipid composition of the epidermis in cultured **skin substitutes** in vitro and after grafting to athymic mice. The cultured **skin substitutes** were prepared from human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates. After 14 days of incubation, cultured **skin substitutes** were grafted orthotopically onto full-thickness wounds in athymic mice. Samples for lipid analysis were collected after 14 and 34 days of in vitro incubation, and 3 weeks and 4 months after grafting. Both in vitro samples show disproportions in epidermal lipid profile as compared with the native human epidermis, i.e., a low amount of phospholipids (indicating imbalance in proliferation and differentiation): a large excess of triglycerides (storage lipids): and low levels of free fatty acids, glucosphingolipids, cholesterol sulfate, and **ceramides** - suggesting abnormal composition of stratum corneum barrier lipids. Fatty acid analysis of cultured **skin substitutes** in vitro revealed insufficient uptake of linoleic acid. which resulted in increased synthesis of and substitution with monounsaturated fatty acids, mainly oleic acid. These abnormalities were partially corrected by 3 weeks after grafting; and 4 months after grafting, all epidermal lipids, with some minor exceptions, were synthesized in proportions very similar to human epidermis. Results of this study show that grafting of cultured **skin substitutes** to a physiologic host permits the recovery of lipid in proportion to that required for barrier formation in normal human epidermis.
- L5 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
 AU Michel, Martine; Germain, Lucie; Belanger, Pierre Maxime; Auger, Francois A.
 TI Functional evaluation of anchored **skin equivalent** cultured in vitro: percutaneous absorption studies and lipid analysis
 SO Pharmaceutical Research (1995), 12(3), 455-8

CODEN: PHREEB; ISSN: 0724-8741

AB An original type of **skin equiv.** (SE), produced by a procedure involving seeding keratinocytes on various dermal substrates, is proposed that can be easily prepd. and manipulated for permeability studies. The authors demonstrated a relationship between lipid type, content and percutaneous absorption rate in anchored **skin equiv.** (ASE), in which fibroblasts are included in the SE prepn. Because of similar lipid profiles, the qual. percutaneous absorption properties were comparable to those of normal human and mouse skin. However, the lower **ceramide** d. in ASE epidermis likely explained its higher permeability. Cell culture conditions could be further investigated to increase lipid amt. in order to approach intact skin barrier properties.

L5 ANSWER 16 OF 20 MEDLINE on STN DUPLICATE 7

AU Auger F A; Lopez Valle C A; Guignard R; Tremblay N; Noel B; Goulet F; Germain L

TI **Skin equivalent** produced with human collagen.

SO IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY. ANIMAL, (1995 Jun) 31 (6) 432-9.

Journal code: 9418515. ISSN: 1071-2690.

AB Several studies have recently been conducted on cultured **skin equivalent** (SE), prepared using human keratinocytes seeded on various types of dermal equivalents (DE). We previously showed the advantages of our anchorage method in preventing the severe surface reduction of DE due to fibroblast contractile properties in vitro. A new anchored human SE was established in our laboratory in order to obtain a bioengineered tissue that would possess the appropriate histological and biological properties. In order to compare the effects of different collagen origins on the evolution of SE in vitro, human keratinocytes were seeded on three types of anchored DE. A comparative study was carried out between bovine SE (bSE), human SE (hSE), and human **skin equivalent** containing additional dermal matrix components (hSE+). Immunohistological analysis showed that hSE and hSE+ presented good structural organization, including the deposition of several basement membrane constituents. Higher amounts of transglutaminase, **ceramides**, and keratin 1 were detected in the epidermal layers of all SE when cultured at the air-liquid interface. However, a 92 kDa gelatinase activity was higher in bovine **skin equivalent** (bSE) compared to hSE cultures. The use of human collagens comparatively to bovine collagen as SE matricial component delayed the degradation of the dermal layer in culture.

L5 ANSWER 17 OF 20 MEDLINE on STN DUPLICATE 8

AU Fartasch M; Ponc M

TI Improved barrier structure formation in air-exposed human keratinocyte culture systems.

SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1994 Mar) 102 (3) 366-74.

Journal code: 0426720. ISSN: 0022-202X.

AB The epidermis (including stratum corneum) of human keratinocytes cultured at the air-liquid interface attached to an appropriate substrate shows a morphology closely mimicking that of its in vivo counterpart. In spite of the histologic similarities, the barrier function seems to be impaired. The aim of the present study was to characterize development and structure of the epidermal permeability barrier in two human skin recombinants using electron microscopy (including ruthenium tetroxide-post fixation technique) and analysis of lipid composition. The epidermis was reconstructed by growing human keratinocytes either on de-epidermized dermis or on a bovine collagen-containing matrix with active fibroblasts (**Living Skin Equivalent**). Ultrastructurally both culture systems showed a) an abnormal lamellar body delivery system, b) disturbance of transformation into lamellar lipid bilayers, c) an impaired structural organization and distribution of the epidermal lipids in the intercellular spaces. In either of the systems used, prolongation of the

culture period did not induce any significant improvement in the stratum corneum lipid organization. Whereas the Living **Skin Equivalent** showed only sparse lamellar bodies, the number of lamellar bodies in the human keratinocyte culture on de-epidermized dermis grown in regular medium seemed to be comparable to native skin. Contrary to the Living **Skin Equivalent**, the keratinocyte culture on de-epidermized dermis contained a higher number of intracorneocytic lipid droplets correlating with a higher triglyceride content in the lipid analyses. By reconstructing the keratinocyte culture on de-epidermized dermis with the same medium as used for the Living **Skin Equivalent**, both lipid composition (lower triglyceride, higher **ceramide** contents) and structural organization were improved, and regular lamellar lipid bilayers comparable to those of native skin appeared.

- L5 ANSWER 18 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 9
- AU Nolte, C. J. M. (1); Oleson, M. A.; Bilbo, P. R.; Parenteau, N. L.
TI Development of a stratum corneum and barrier function in an organotypic
skin culture.
- SO Archives of Dermatological Research, (1993) Vol. 285, No. 8, pp. 466-474.
ISSN: 0340-3696.
- AB The stratum corneum of human skin is responsible for maintaining the
epidermal permeability barrier. We have developed a bilayered skin culture
(SC) which forms a corneum 35 +/- 1 cell layers thick 21 days after being
raised to the air-liquid (A/L) interface. By the 7th day after raising to
the A/L interface the corneocytes were irregularly shaped and had
cross-sectional areas (CSA) of $300 \mu\text{m}^2$. By the 21st day the
corneocytes had assumed polygonal shapes and had a CSA (100-250 μm^2)
similar to that of human foreskin. The total lipid (TL) content of the
corneum averaged 5-7% of the lyophilized weight. **Ceramide**
content increased from 20% of TL at day 7 of A/L interface culture to 30%
at day 21. Triglycerides decreased from 43% to 17% of TL during the same
period. Free fatty acids comprised 5.5% of TL at day 21 of A/L interface
culture. The intercorneocyte spaces contained stacks of lipid lamellae.
However, the stacks lacked the Landmann unit repeat. Abnormal lamellar
structures were observed in both the intra- and extracorneocyte spaces.
Transepidermal water loss (TEWL) was $> 4 \text{ mg/cm}^2 \text{ per h}$ throughout the
culture period. Lipid supplementation of the culture medium and culturing
in a low humidity environment improved barrier function by 50%. However,
the effects were not additive. The SC developed a near-normal corneum, but
did not achieve barrier competence, due at least partially to
abnormalities in lipid composition and organization. Improvement of
barrier function with lipid supplementation or low humidity indicates that
modifications of the culture environment may facilitate the SC in
assembling a permeability barrier **equivalent** to human
skin.
- L5 ANSWER 19 OF 20 MEDLINE on STN DUPLICATE 10
- AU Boyce S T; Williams M L
- TI Lipid supplemented medium induces lamellar bodies and precursors of
barrier lipids in cultured analogues of human skin.
- SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1993 Aug) 101 (2) 180-4.
Journal code: 0426720. ISSN: 0022-202X.
- AB Barrier function of cultured **skin substitutes** (CSS) is
required for their effective use in clinical treatment of skin wounds, and
for percutaneous absorption in vitro. Arachidonic, palmitic, oleic, and
linoleic free fatty acids, in conjunction with the antioxidant
alpha-tocopherol acetate (lipid supplements, "LS"), were added to nutrient
media of CSS to provide precursors of epidermal barrier lipids. CSS were
composed of human keratinocytes (HK), fibroblasts (HF), and
collagen-glycosaminoglycan substrates, and were incubated for 14 d
submerged or lifted to the air-liquid interface in media based on MCDB 153
+/- LS. Duplicate samples (30 cm²) were harvested and the epidermal

analogue was analyzed for total protein, total DNA, total lipid, lipid fractions including acylglucosylceramide (AGC), and presence of lamellar bodies. Significant increases ($p < 0.05$) were detected between CSS incubated in +LS medium for total lipid, total DNA, **ceramide**, glucosylceramide, triglycerides, and diglycerides. AGC and lamellar bodies were detected only in epithelia of CSS incubated in +LS medium. These data show that free fatty acids, vitamin E, and lifting of CSS promote increased epithelial morphogenesis compared to CSS cultured submerged without lipid supplements. Presence of lamellar bodies and AGC suggests enhanced production in vitro of barrier-associated epidermal lipids.

- L5 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
AU Ponec, M.
TI Reconstruction of human epidermis on de-epidermized dermis: expression of differentiation-specific protein markers and lipid composition
SO Toxicology in Vitro (1991), 5(5-6), 597-606
CODEN: TIVIEQ; ISSN: 0887-2333
AB By using the Regnier-Prunieras method for the reconstruction of epidermis in vitro, a multilayered epidermis was obtained with an overall structure resembling that of native human epidermis. Evaluation of the expression and localization of a no. of differentiation-specific protein markers revealed that human epidermis reconstructed on de-epimerized dermis shares some common features with hyperproliferating epidermis, such as the expression of keratin 6, and of involucrin, transglutaminase, and filaggrin in suprabasal layers. Analyses of lipid content revealed that the air-exposed keratinocyte cultures reproduce to a high degree the lipids of the native epidermis, with the exception of higher triglyceride and lower glycosphingolipid content and a very low content of linoleic acid. The differences obsd. in the expression of differentiation-specific protein markers, as well as in the lipid compn., can be most probably attributed to the culture conditions used, since on culturing freshly excised skin under the air-exposed conditions similar deviations from the non-cultured tissue were obsd. as in the reconstructed epidermis. By using [^{14}C]linoleic acid and [^{14}C]acetate it was found that the air-exposed human keratinocyte cultures are capable of synthesizing all lipid species that are present in the native tissue. However, some lipid species are synthesized at rates that are different from those in vivo. This may explain the differences obsd. in the bulk lipid compn. between reconstructed epidermis and its in vivo counterpart.

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L5 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:696151 CAPLUS
DN 137:237786
TI Improved **skin substitutes** and uses thereof
IN Comer, Allen; Allen-Hoffmann, Lynn; Hoffmann, Michael; Ivarie, Cathy
Ann-Rasmussen; Conrad, Paul Barth
PA Stratatech Corporation, USA
SO PCT Int. Appl., 104 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002070729	A2	20020912	WO 2002-US6088	20020301
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2002164793	A1	20021107	US 2002-87641	20020301
	US 2002168768	A1	20021114	US 2002-87346	20020301
	US 2002187498	A1	20021212	US 2002-87388	20020301
PRAI	US 2001-273034P	P	20010302		
	US 2001-287898P	P	20010501		

L5 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:51225 CAPLUS
DN 136:123381
TI Cosmetic composition comprising a **ceramide** precursor for improving natural or reconstructed epidermis, resulting **skin equivalent**
IN Philippe, Michel; Castiel, Isabelle; Ferraris, Corinne; Arbey, Eric
PA L'oreal, Fr.
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002003944	A1	20020117	WO 2001-FR1800	20010611
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
	FR 2811556	A1	20020118	FR 2000-9061	20000711
	FR 2811556	B1	20020906		
	EP 1303252	A1	20030423	EP 2001-943594	20010611
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			
PRAI	FR 2000-9061	A	20000711		

WO 2001-FR1800 W 20010611

OS MARPAT 136:123381

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